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Activity of Ceftaroline against *Enterococcus faecium* PBP5

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The opportunistic human pathogen *Enterococcus faecium* overproduces the low-affinity PBP5. In clinical strains, mutations in PBP5 further reduce its acylation rate by β-lactams. Previous studies have reported that ceftaroline had poor inhibitory activity against β-lactam-resistant *E. faecium* strains. In this study, we show that ceftaroline exhibits killing activity against our laboratory-derived ampicillin-resistant *E. faecium* mutant that overproduces a wild-type PBP5 and that ceftaroline inactivates PBP5 much faster than benzylpenicillin and faster than ceftobiprole.

β-Lactam antibiotics (penicillins, cephalosporins, carbapenems, and monobactams) represent the most important group of drugs prescribed to treat bacterial infections. They form stable acyl-enzymes with their targets, the membrane-bound D, D-transpeptidases, which are essential enzymes in peptidoglycan biosynthesis. These proteins are usually referred to as penicillin-binding proteins (PBPs) (1–3). The presence or overproduction of lower-affinity PBPs is responsible for the resistance of Gram-positive cocci against β-lactam antibiotics. It is known that the opportunistic human pathogen *Enterococcus faecium* overproduces the low-affinity PBP5 and that mutations further reduce the acylation rate of PBP5 by altering its amino acid sequence (4, 5). Ceftaroline is a cephalosporin with broad-spectrum activity against Gram-positive organisms, including methicillin-resistant *Staphylococcus aureus* (MRSA), and many common Gram-negative bacteria except those producing extended-spectrum β-lactamase (ESBL) or AmpC β-lactamase (6–8). The *Staphylococcus aureus* low-affinity PBP2a is closely related to PBP5 (9). It is inhibited by ceftaroline, the active metabolite of the prodrug ceftaroline fosamil, which was recently approved by the U.S. Food and Drug Administration (FDA) for use in adult patients with acute bacterial skin and skin-structure infections and community-acquired bacterial pneumonia and more recently approved by the European Medicines Agency (EMA) for similar indications (10, 11). Previous studies have reported that ceftaroline had poor inhibitory activity against β-lactam-resistant *E. faecium* strains, all of which were expected to possess a PBP5 protein (6, 12). To better understand these differences, we have characterized the interaction between a wild-type form of PBP5 and ceftaroline.

Ceftaroline MIC values determined by the microdilution method (13) for *E. faecium* D63r and D63 strains (5) were 2 and 0.25 mg · liter^{-1}, respectively. These values contrasted with those reported by others (10–12), who have found that most ampicillin-resistant *E. faecium* clinical isolates were resistant to ceftaroline and concluded that ceftaroline had little activity against most isolates of *E. faecium*. The killing effects (14) of ceftaroline on *E. faecium* D63 and D63r strains were studied by exposing exponentially growing cultures of both strains to increasing concentrations of antibiotics corresponding to 1 and 4 times their respective MICs (Fig. 1). Ceftaroline showed killing effects (1 log drop after 24 h) for concentrations higher than the MICs. We determined the 50% inhibitory concentration (IC_{50}) values of ceftaroline, benzylpenicillin, cefepime, and ceftazidime on the various PBPs of *E. faecium* D63 and D63r by using purified membrane preparations and fluorescent ampicillin (5, 15). Titration of the PBPs by

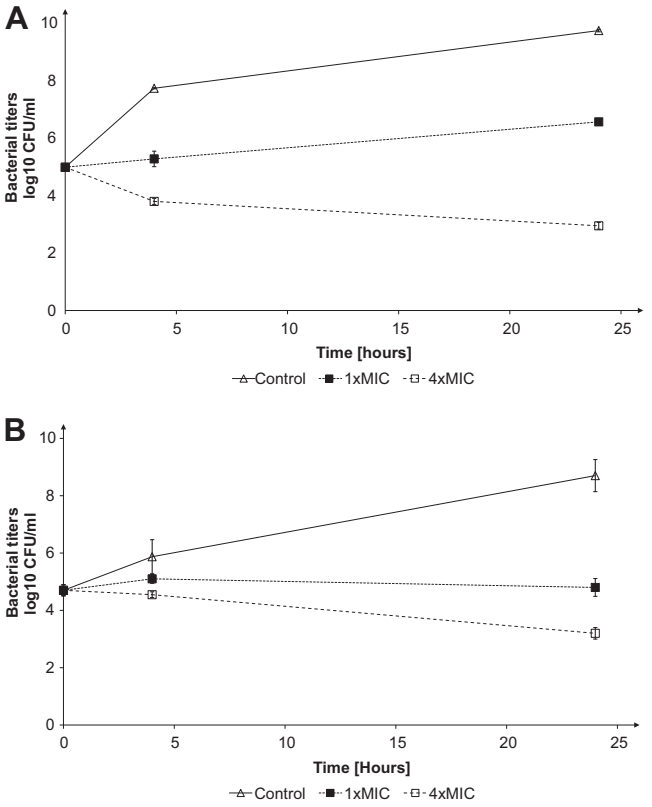

FIG 1 Drug binding studies for ceftaroline with D63 and D63r membrane fractions. MICs of D63 (A) and D63r (B) for ceftaroline were 0.25 mg/liter and 2 mg/liter, respectively. The surviving bacteria were counted after 0, 4, and 24 h of incubation at 37°C by subculturing serial dilutions (at least 10-fold, to minimize drug carryover). For all data, the standard deviations were below 10% of the mean.

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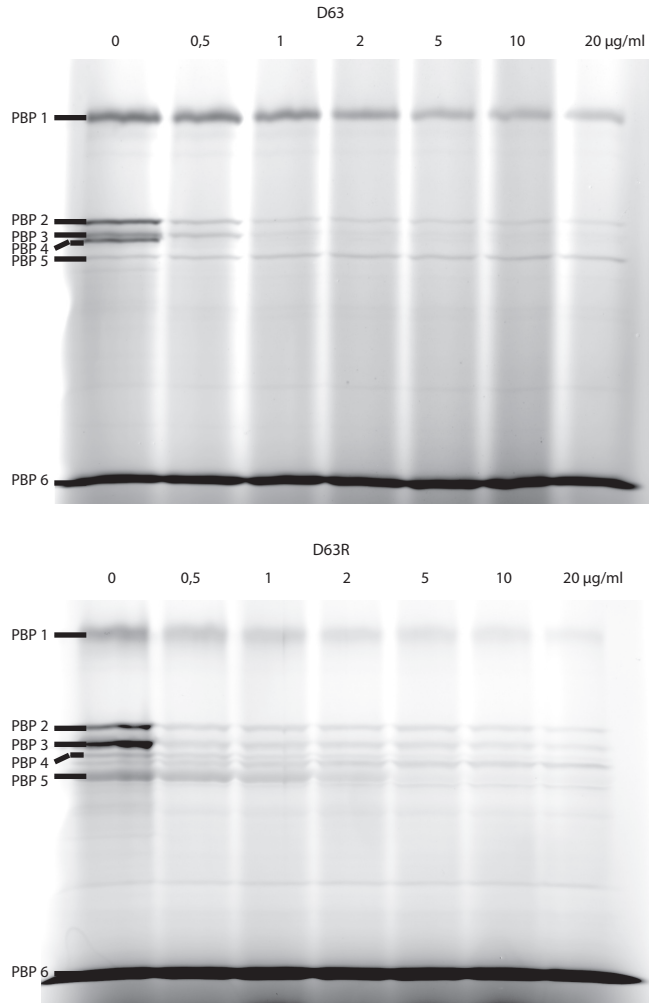

FIG 2 PBP binding studies for ceftaroline with membrane fractions from D63 and D63r. The concentration of ceftaroline in the competition assay with fluorescent ampicillin is indicated in μg/ml.

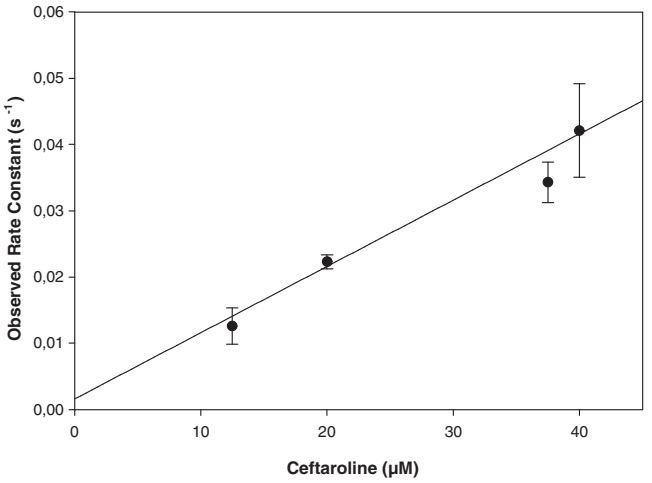

FIG 3 Variation of the pseudo-first-order inactivation rate constant (k_a) as a function of ceftaroline concentration. The opening of the ceftaroline β-lactam ring due to the acylation reaction was monitored by recording the decrease of absorbance at 300 nm ($\Delta\varepsilon = 4{,}330\ M^{-1}\ cm^{-1}$) with a Specord 200 spectrophotometer (Analytik Jena, Germany), at 30°C in 10 mM phosphate buffer (pH 7.0) with 10 μM sPBP5.

ceftaroline (Fig. 2 and Table 1) revealed that at twice the MIC values, all high-molecular-mass PBPs were inhibited by the antibiotic (Fig. 2) and that the low-molecular-mass PBP6, a D, D-carboxypeptidase (16), was not affected. This inhibition pattern is completely different from that obtained with benzylpenicillin, for which PBP5 is the most resistant PBP (17). The IC_{50}s for ceftaroline on PBP5 were 1.40 ± 0.09 mg/liter and 0.7 mg/liter when determined with membrane preparations and the purified sPBP5, respectively. The kinetic parameters governing the acylation of PBP5 by ceftaroline were determined by using the sPBP5 (a soluble PBP5 from which the N-terminal membrane anchoring peptide was removed), which was overproduced and purified as previously described, except that the molecular sieve step was eliminated (18). The kinetics model used has been described previously (17). On the basis of the data shown in Fig. 3, the second-order rate constant k_{+2}/K for ceftaroline was 950 ± 70 $M^{-1}\ s^{-1}$, i.e., 50 to 100 times higher than the value reported for benzylpenicillin (15 to 24 $M^{-1}\ s^{-1}$), which indicates that ceftaroline inactivates sPBP5 much faster than benzylpenicillin (5) and faster than ceftobiprole, another anti-MRSA cephalosporin (k_{+2}/K = 110 ± 10 $M^{-1}\ s^{-1}$ [13]). While the inhibitory activity of ceftaroline for *E. faecium* PBP5 is significant, the k_{+2}/K rate constant for sPBP5 is 15 to 25 times lower than those determined for MRSA PBP2a (23,600 ± 2,000 $M^{-1}\ s^{-1}$ [unpublished data]) produced and purified in our laboratory (19) and for the penicillin-resistant *Strep-*

TABLE 1 Inhibition of PBPs from *E. faecium* D63 and D63r strains

Strain	Antibiotic	IC_{50}[a] (mg/liter) for PBP:						MIC (mg/liter)
		1	2	3	4	5	6	
D63r	Benzylpenicillin	1.6 ± 0.4	0.06 ± 0.01	0.6 ± 0.3	8 ± 5	55 ± 15	2 ± 1	70
	Cefepime	1.7 ± 0.6	0.6 ± 0.3	0.5 ± 0.1	>200	>200	>50	>100
	Ceftazidime	0.9 ± 0.6	0.5 ± 0.3	0.04 ± 0.01	>200	>200	>50	>100
	Ceftobiprole	0.7 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	1.8 ± 0.2	1 ± 0.2	>16	8
	Ceftaroline	0.63 ± 0.09	0.07 ± 0.01	0.06 ± 0.01	0.97 ± 0.03	1.38 ± 0.1	>50	2
D63	Benzylpenicillin	0.9 ± 0.7	0.1 ± 0.07	1.3 ± 0.6	10 ± 8	75 ± 25	1.5 ± 1	5
	Ceftobiprole	0.6 ± 0.2	0.2 ± 0.1	0.6 ± 0.2	1.5 ± 0.3	0.7 ± 0.1	>16	2
	Ceftaroline	0.64 ± 0.04	0.07 ± 0.01	0.06 ± 0.01	0.212 ± 0.03	0.53 ± 0.03	>50	0.25

[a] Concentration of β-lactam antibiotic that reduced binding of fluorescent ampicillin by 50% compared to a control containing no drug. IC_{50}s for D63r PBP5 against antibiotics other than ceftaroline were previously published (17).

tococcus pneumoniae PBP2x (12,600 M^{-1} s^{-1}) (20). The sPBP5-ceftaroline adduct was very stable. Indeed, no free enzyme could be detected after a 4-hour incubation of the isolated acyl-enzyme at 37°C.

We have shown that ceftaroline inactivates the low-affinity *E. faecium* PBP5 more efficiently than benzylpenicillin. It exhibits bacterial killing against our laboratory-derived ampicillin-resistant *E. faecium* mutant that overproduces the wild-type PBP5. Such a profile differs from those of most *E. faecium* clinical isolates, which were reported as resistant to all β-lactams, including ceftaroline (6). Like other cephalosporins, ceftaroline is not indicated for treatment of enterococcal infections. It is likely that most *E. faecium* clinical isolates produce mutant PBP5 forms with reduced affinity. This suggests that simple overexpression of wild-type PBP5 is sufficient to moderately increase the MIC for ceftaroline but that amino acid substitutions in the protein are necessary to confer high-level resistance. Among the β-lactams tested to date, ceftaroline has the highest affinity for wild-type PBP5, which makes it a potentially useful tool for PBP5 studies.

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